PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
PETER F. CORLESS
EDWARDS & ANGELL, LLP
P.O. BOX 55874
BOSTON, MA 02205

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	31 JAN 2006
Applicant's or agent's file reference 60677 (49163	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US05/07631	10 March 2005 (10.03.2005)	10 March 2004 (10.03.2004)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): A01N 63/00; C07K 1/00; A01K 67/00 and US Cl.: 424/93.1; 530/350; 800/8

Applicant

UNIVERISTY OF FLORIDA

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450	28 December 2005 (28.12.2005)	Ram R Shukla Telephone No. 571-272-1600
Facsimile No. (571) 273-3201		

Form PCT/ISA/237 (cover sheet) (April 2005)

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-13, 16, 17, 22-24, 27-29	YES
	Claims 14, 15, 18-21, 25, 26	NO
Inventive step (IS)	Claims 5-7,16,17,22-24 and 27-29	YES
	Claims 1-4,8-15, 18-21, 25 and 26	NO
Industrial applicability (IA)	Claims 1-29	YES
	Claims NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:
Claims 14, 15, 18-21, 25 and 26 lack novelty under PCT Article 33(2) as being anticipated by Kanadia et al. Kanadia teaches a transgenic mouse comprising a deletion of exon 3 of the endogenous Mbnl1 gene (page 1978, Figure 1). Kanadia also teaches that said mouse exhibits at least one symptom of myotonic dystrophy, namely myotonia and ocular cataracts (page 1979, col. 3, paragr. 2, lines 1-2 and Figure 2); however, Kanadia teaches that said mouse does not develop neonatal muscle weakness that is typically associated with congenital DM1 in humans (page 1980, col. 3, paragr. 1, lines 1-3). Kanadia teaches that the symptoms of said mouse are indicative of a microsatellite repeat expansion disease (i.e. DM types 1 and 2) caused by a microsatellite expansion in a non-coding region of DNA (i.e. within the 3' UTR of the DMPK gene for DM type 1 and within the first intron of the ZNF9 gene for DM type 2; page 1979, col. 1, lines 1-9). Kanadia teaches immunoblot analysis of total spleen protein and thus teaches a cell (i.e. spleen cell) isolated from said mouse (page 1978, Figure 1D). Kanadia teaches that said mouse exhibits abnormal muscleblind proteins in that the Mbnl1 protein is not expressed in the spleen of said mouse (page 1978, Figure 1D). Kanadia teaches that said mouse exhibits abnormal splicing of Clcn1 mRNA resulting in Clcn1 mRNA encoding non-functional ClC-1 protein (page 1979, col. 3, paragr. 2, line 1 to page 1980, col. 1, line 6). Kanadia also teaches that said mouse exhibits similar abnormal splicing of Tnnt2 and Tnnt3 mRNA (page 1980, col. 2, paragr. 1).

Claims 1-4 and 8-13 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Miller and Hartigan-O'Connor. Kanadia does not teach a method of treating a disease associated with aberrant microsatellite expansion comprising administering nucleic acid encoding Mbnl1 and does not teach a pharmaceutical composition comprising recombinant adeno-associated virus containing a transgene that encodes Mbnl1. Miller teaches a model for DM1 wherein microsatellite-containing mutant DMPK mRNA sequesters MBNL protein from its normal RNA-binding sites (page 4446, col. 1, lines 6-11 and page 4445, Figure 7). Miller further teaches that a causative agent in DM1 may be "sequestration of (CUG)n-binding proteins (i.e. MBNL proteins) on mutant DMPK RNAs and depletion from other transcripts that require these proteins for normal gene expression" (page 4440, col. 1, lines 4-8). It would have been obvious to one of ordinary skill in this art to combine the teachings of Kanadia and Miller to provide the claimed method of treating a disease associated with aberrant microsatellite expansion comprising administering nucleic acid encoding MBNL protein. Further, it would have been obvious to one of ordinary skill in this art to formulate a pharmaceutical composition of a nucleic acid encoding MBNL protein for use in said method by constructing a recombinant adeno-associated virus (rAAV) containing a transgene that encodes MBNL protein. It was well known in this art at the time of the invention that rAAV could be used in pharmaceutical compositions to practice gene therapy. For example, Hartigan-O'Connor teach the benefits of rAAV vectors in general (page 230, col. 1, paragr. 3 to page 231, col. 1, line 2) and specifically in relation to delivery of therapeutic genes to dystrophic muscles (page 225, col. 2, line 1 to page 227, col. 2, paragr. 2 and see entire document). In summary, it would have been obvious to one of ordinary skill in this art to combine the teachings of Kanadia, Miller and Hartigan-O'Connor to provide the claimed method of treating a disease associated with aberrant microsatellite expansion comprising administering a rAAV containing a transgene encoding MBNL proteins.

Claims 5-7, 16, 17, 22-24 and 27-29 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest: the claimed method of treatment wherein treating comprises reversing the misplicing of the genes encoding amyloid beta precursor protein, NMDA receptor or microtubule associated protein tau; a transgenic mouse comprising a deletion of exon 3 of the

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

endogenous Mbnl1 gene, wherein said mouse exhibits symptoms typical of a disease associated with aberrant microsatellite expansion in humans, wherein the symptoms of said mouse comprise muscle weakness and ocular cataracts, wherein the disease associated with aberrant microsatellite exapansion in humans is caused by a microsatellite expansion in a coding region of DNA, wherein said mouse exhibits loss of functional amyloid beta precursor protein, NMDA receptor or microtubule-associated protein tau; or a method of using a transgenic mouse comprising a deletion of exon 3 of the endogenous Mbnl1 gene for screening compounds useful in the treatment of diseases associated with aberrant microsatellite expansion.

Claims 1-29 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.